Fluorescence Spectroscopy I

733-Pos Board B533
Global Analysis of Time-Resolved Anisotropy from Multiple Probes in a Rigid Globular Protein
Dmitri Toptygin, Ludwig Brand, Gregory S. Chirikjian.
Rotational diffusion of a rigid globular protein is described by a second-rank tensor (a 3x3 matrix). In the reference frame aligned with the principal axes only the three diagonal elements are non-zero. In the general case one must assume that all three principal diffusion coefficients are different. The knowledge of the three coefficients makes it possible to estimate the protein shape. The aim of this work is determining the three principal diffusion coefficients and the approximate shape of a protein from the time-resolved fluorescence anisotropy data.
We developed from basic principles the theory of polarized fluorescence for naturally occurring fluorophores within rigid proteins. Our theory does not assume that the excited-state population decay is monoexponential or that the exciting wavelength is in resonance with a single electronic transition. Thus, our model is applicable to tryptophan residues. In the special case of monoexponential fluorescence and a single electronic transition our model becomes equivalent to that of Chuang and Eisenthal *J. Chem. Phys.* **57,** 5094 (1972).
For an arbitrary-shape rigid protein the time-resolved anisotropy is a linear combination of five exponentials that cannot be resolved if the rate constants are treated as free fitting parameters. In our model the five rate constants are functions of only three principal diffusion coefficients, which play the roles of the global fitting parameters. Furthermore, we simultaneously analyze data sets obtained using two exciting wavelengths (varying ^1L_a and ^1L_b excitation) and multiple single-tryptophan-containing variants of the same protein. The preexponential amplitudes vary as a function of the directions of ^1L_a and ^1L_b transitions for each variant. In principle, this makes it possible to find both the shape of the protein and the probe orientations.

734-Pos Board B534
Extracting Protein Interaction Information from FRET Data: A Study with Bayesian Inference and Simulations
Catherine A. Lichten, Peter Swain.
Advances in fluorescence microscopy have made it possible to collect increasingly sophisticated experimental data, but equally important is developing analysis techniques to extract information from this data. One experimental tool with potential to yield further useful quantitative information is Förster resonance energy transfer (FRET), which is frequently used to probe interactions between proteins. We hypothesized that applying Bayesian inference to analyze FRET data would allow us to create a flexible method, capable of estimating both the dissociation constant (Kd) and the FRET efficiency (Efr) by producing the distribution of values that is statistically consistent with a given dataset. The goal of this work is to develop such a method.
We designed an algorithm that uses a Markov Chain Monte Carlo method to explore possible values of Kd, Efr and other parameters, seeking those that make experimental observations be consistent with predictions from a mathematical model of FRET. The algorithm can incorporate uncertainty in experimental measurements and calibration, allowing us to systematically explore how measurement errors, numbers of repeated measurements and fluorophore concentrations impact the quality of the estimate. It also has the flexibility to incorporate additional knowledge about the system that may improve the estimate, such as fluorescence lifetime data.
Applying our method to data from computer simulations, we demonstrate how variations in concentrations of fluorophores and the quality and quantity of the data determine how much can be inferred about the underlying parameters of interest. Overall, our work has applications for extracting additional quantitative information from FRET data and for optimally designing FRET experiments to measure protein interaction strength.

735-Pos Board B535
Thioamides as Fluorescence Quenchers: Minimalist Chromophores to Monitor Protein Dynamics
Jacob M. Goldberg, Solongo Batjargal, Alyssa M. Klein, E. James Petersson.
Reducing the size of spectroscopic probes can increase the spatial resolution of fluorescence experiments on protein dynamics. We have shown *p*-cyanophenylalanine (Cnf) and backbone thioamides to be a fluorophore/quencher pair and we have determined their working distance to be 8-30 Å. We have used this pair to study the thermal denaturation of a Cnf/thioamide-labeled version of villin headpiece by fluorescence spectroscopy. We are currently exploring quenching interactions with other fluorophores and applying the method to monitor binding and folding in a few benchmark proteins. The small size of the thioamide probe opens the possibility of mapping conformational changes with a density far beyond what is currently possible.

736-Pos Board B536
MD Simulations and FRET Studies of Dye-Labeled RNA
Peker Milas, James Moore, Benjamin D. Gamari, Brent P. Krueger, Lori S. Goldner.
Fluorescence resonance energy transfer (FRET) is a powerful technique for understanding the structural transformations of RNA, DNA and proteins. With a few notable exceptions, the contribution of fluorophore and linker dynamics to these FRET measurements has not generally been investigated. Towards a better understanding of FRET on dye-labeled RNA, we present molecular dynamic (MD) simulations of 16mer double-stranded RNA with cyanine dyes attached at either the 3' or 5' ends with a 3 carbon linker. Water is included explicitly, and both dyes are in the ground state configuration. Differences in these two labeling strategies are discussed. We compare our simulations to data taken both on surface-attached and droplet-confined molecules. The effect of relative dye orientation and distance fluctuations due to the flexible linker are explicitly investigated.

737-Pos Board B537
Frequency Domain and Time Domain One Photon and Super-Continuum Micro Luminometry on RU and TB Complexes
M. van de Ven.
1
Ruthenium and lanthanide metal complexes can show very long microsecond - millisecond lifetimes increasing with their state of ligation and dependent on the concentration of quenchers like oxygen. They exhibit sharp spectral peaks and may show a strong luminescence enhancement. These features are used to an advantage in biosensor development, medical imaging, cancer treatment and environmental monitoring. Under oxic and anoxic conditions luminescence characteristics were explored at high acetate buffer concentration at room temperature.
One-photon Light Emitting Diodes, laser diode lightsources and a supercontinuum laser were attached to an ISS Alba FastFLIM-FRET setup, a ChronosBH for Time Correlated Single Photon Counting and a ChronosFD multi-frequency phase and modulation luminometer. Combinations of computer-controlled filterwheel, excitation monochromator and polarizer with half-waveplate allowed automated wavelength selection and intensity adjustment. Magic angle conditions prevailed throughout. Hamamatsu H7422P-40 detector signals were processed by Becker & Hickl PMS-400A and SPC-130 cards. Frequency-domain results are compared with time-domain analysis as obtained with classical fluorescence and phosphorescence decay parametrization and using the phasor approach. Merits towards incorporation into biosensor applications of either method and effects of quenching, photobleaching and buffer background are discussed.

738-Pos Board B538
Fluorescent Labels in Proteins: How Random is Random Labeling
Qiaoqiao Ruan, Cheng Zhao, Sergey Y. Tetin.
Most proteins have one or more lysine residues exposed on their surface that can react with fluorescent probes directed to ϵ-amino groups. If a protein contains multiple lysine residues, labeling is often considered random assuming equal probability of the probe to interact with any of available lysines. Working with human Neutrophil Gelatinase Associated Lipocalin (NGAL), we noticed that the fluorescence of randomly labeled protein is strongly quenched even at low incorporation of the probe (AlexaFluor488 sulfodichlorophenol ester). According to X-ray data NGAL contains sixteen lysine residues, eleven of which are exposed to the solvent. To understand the labeling and quenching mechanisms, we performed labeling at multiple probe-to-protein ratios and different pH. The dye incorporation was determined by both absorption spectra and mass spectrometry. Positions of the dye's attachments were identified by protein enzymatic fragmentation followed by mass spectrometric characterizations. Surprisingly, at low incorporation ratios, the probe was always attached to either of two lysine residues, Lys 126 and 135, which are located in the calyx of NGAL in close proximity to Trp 32 and Trp 80. This finding may explain why the fluorescence of labeled NGAL is greatly quenched. At incorporation ratios 6:1 and greater the probe was predominantly linked to five lysines: Lys 31, 63, 126, 135 and 150. The activity of lysine residues at all other positions is much lower. Thus, our study shows that labeling of NGAL is not random and depends on the local microenvironment of targeted ϵ-amino groups.